degrees of maturity of the erythroblasts, and thereby] whereby the erythroblasts are classified into at least two groups according to the degrees of maturity thereof.

Claim 12, line 9, remove the term --of-- between "The method" and "according to claim 11."

Please add the following new claim:

13. The method according to claim 4 wherein the integrity of leukocytes is about 400 mOsm/Kg.H_2O to about 600 mOsm/Kg.H_2O.

REMARKS

This is in response to the Office Action mailed July 3, 2000. In that Office Action the Examiner rejected claims 1-12 under 35 U.S.C. Section 112, second paragraph, as allegedly being indefinite. Moreover, the Examiner rejected claims 1-12 under 35 U.S.C. Section 103(a) as being allegedly unpatentable over U.S. Letters Patent No. 5,047,321 issued to Loken, et al. in view of U.S. Letters Patent No. 5,559,037 issued to Kim et al., and U.S. Letters Patent No. 5,298,426 issued to Inami, et al. Applicants respond to each of the grounds of rejection raised in turn as follows:

Section 112, Second Paragraph.

Claim 1 has been amended to better describe the invention by discriminating between erythroblasts and leukocytes in the hematologic sample based on differences in a two-dimensional distribution chart. Support for this amendment is found throughout the specification, in particular at page 14, line 14 to page 15, line 5. No new matter has been added.

Claim 3 has been amended to better describe the fluorescent leukocyte of step (i) and describing what CY5 dye actually means. No new matter has been added.

Claim 4 has been amended to better describe step (ii) by adding a buffer. Support for these amendments is found at page 12, lines 4-13 of the specification. No new matter has been added. New claim 13 has been added to reflect the preferred integrity of leukocyte range. Support for this claim is also found at page 12, lines 7-10.

Claim 10 has been amended to better describe the invention. No new matter has been added. Support for the amendment to claim 10 appears on Figure 8 and in Example 3, at page 21, line 21 to page 22, line 6.

Claim 12 has been amended to better describe the invention. The amendment is clerical in nature and requires no reference to support.

In the Office Action, claims 1-12 were rejected under 35 U.S.C. § 112, second paragraph. In particular, the Examiner stated that claims 1-12 were vague and indefinite in that the terms "staining" and "labeling" are used interchangeably by the applicants but the two terms have different meanings. By way of this amendment claim has been amended so as to show that the stain binds to the leukocytes so as to better describe the invention. In addition, claim 1 has been amended to better describe the way in which erythroblasts are discriminated from leukocytes using a two-dimensional distribution chart. This amendment obviates the rejection under 35 U.S.C. § 112, second paragraph.

In this regard, claims 2-12 depends from claim 1 and therefore contains all of the features and attributes of claim 1. *In re Fine*, 5 USPQ2d 1596, 1600 (Fed. Cir. 1988). In view of the foregoing, the rejection of claims 1-12 under 35 U.S.C. § 112, second paragraph should be reconsidered and withdrawn.

In the Office Action, the claim 3 was also rejected under 35 U.S.C. § 112, second paragraph as being confusing. By way of this amendment claim 3 has been amended to more definitely define the invention. Claims 10 and 12 have also been amended for clarification.

With respect to claim 10, Applicants respectfully traverses the rejection. Applicants note that the maturity of the erythroblasts determines the degree of dyeing which will result by introducing dye of the given concentration range. Support for this appears at Figure 8, where three kinds of erythroblasts of different maturity dyed with a single nucleotide fluorescent dye (in this instance, propidium iodide) in a concentration of 1mg/L according to Example 3 are classified in stages I to III. By way of this amendment, claim 10 more definitely defines the invention.

In the Office Action claim 4 was rejected under 35 U.S.C. § 112, second paragraph "because it is unclear how the pH (acidic vs. neutral) effects the 'labelling' of the leukocytes." (See Office Action p. 4, 2¶). By way of this amendment claim 4 has been amended to recite a specific pH range as well as a specific osmolarity range.

In view of the foregoing it is respectfully requested that the rejection of claims 1-12 under USC §112, second paragraph be reconsidered and withdrawn.

Section 103(a).

In the Office Action, claims 1-12 were rejected under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 5,047,321 to Loken et al. (herein "Loken") in view of U.S. Patent No. 5,559,037 to Kim et a. (herein "Kim") and U.S. Patent No. 5,298,426 to Inami et al (herein "Inami").

Loken describes a method for multi-parameter analysis of cells in a body fluid sample. The method uses a plurality of fluorescence measurements, comprising at least two nucleic acid dyes and

at least one fluorescent labelled cell surface marker. The method also uses a plurality of light scattering measurements. See Loken, Abstract.

Kim discloses a method for simultaneous and quantitative, flow cytometric analysis of erythroblasts and leukocytes. Kim discloses raising cytoplasmic permeability of nucleotide fluorescent dye. See Kim, abstract.

Inami discloses a method which comprises using a specified dye taken up by erythrocytic nucleated cells so that their nuclei are stained and can be readily differentiated from other cells by measurement with a flow cytometer. The method uses a two-step staining that using a first fluid that is an acidic hypotensive fluorescent dye solution and a second fluid that is a solution that changes the osmosality and pH of the first fluid. See Inami, abstract.

In making the rejection the Examiner relied on Loken as disclosing a method comprising combining a body fluid sample such as whole blood with at least two nucleotide fluorescent dyes such as RNA dye or DNA dye and at least one fluorescent labeled antibody or cell marker to form a labeled mixture. (Office Action, p. 6, ¶ 5). The Examiner contends that the dyes independently and differentially assess different characteristics of nucleated cells in a sample.

The Applicants respectfully disagree. In order to establish a *prima facie* case of obviousness the references cited must teach every element recited in the claims and identify the necessary motivation to combine these elements. In re Rouffet, 149 F. 3d 1350; 47 USPQ2d 1453 (Fed. Cir., 1998). In short, the citations must "suggest the desirability of the combination" that is claimed. See MPEP 2143.01 at 2100-110, 111 and MPEP 2145 (j) 3 at 2100-127. Statements with regard to relevant skill in the art do not suffice to "bridge over gaps in substantive presentation of an obviousness case." Al-Site Corp. v. VSI International, Inc., 174 50 USPQ2d 1161 (Fed. Cir. 1999). It is respectfully submitted that the cited references fail not only to disclose or teach each element of the Applicant's

claims, they also fail to provide the requisite suggestion *to do* what the applicants have done. For these reasons alone, the rejection of the claims is insufficient as a matter of law. Ex parte Levengood, 28 USPQ2d 1300, 1301-02 (BPAI 1993).

Loken fails to teach, suggest or disclose the elements of Applicants' claim 1. Figures 4B and 6B of Loken describe the classification of groups containing erythroblasts. The classified groups, however, also contain other cells, such as, normoblasts, monoyloids or lymphoid precursors. (Col. 10, lns. 23-26). The discrimination mechanism in Loken comprises 1) measuring fluorescences derived from two nucleic dyes and forming a two-dimensional distribution scattergram utilized the two fluorescences as coordinate axes; 2) enclosing a certain region of the distribution scattergram in a window; and 3) measuring orthogonal scattered light and fluorescences corresponding to a labeled antibody to obtain a two-dimensional distribution scattergram utilizing the two lights as the coordinates axes. In other words, the erythroblasts cannot be clearly discriminated from other cells at all, let alone using a two dimensional distribution chart. In addition, Loken fails to teach or suggest "increasing permeability of cytoplasm of specific nucleated cells" as required by the present invention. In fact, Loken does not even mention the discrimination of erythroblasts.

With respect to Kim, although the staining of erythroblasts using a nucleotide fluorescent dye is disclosed, Kim fails to teach, suggest or disclose employing either a dye or fluorescent labeled antibody for staining the leucocytes. Moreover, Kim discloses discriminating erythroblasts by detecting three parameters on one fluorescent light and two kinds of scattered lights, which differs from the method of claim 1 where use of only two parameters (fluorescent lights) is claimed. While Applicant submits that neither Kim nor Loken disclose any motivation or incentive to combine these references, fundamentally Kim demonstrably does not address the deficiencies of Loken.

Moreover, Inami fails to disclose use of two fluorescent light parameters as required by the claims of the present invention. As is clearly evident, the two dimensional charts referenced by the examiner in Inami (figures 9, 10 and 11) plot fluorescence against side scattered light intesity. In addition, Inami fails to disclose staining leucocytes with a fluorescent binding antibody, as required in Claim 1.

All the references fail to disclose each element of Applicants' claim 1, any teaching or suggestion as to combining these references also lacks support. Applicants submit that no teaching, suggestion or disclosure in any of the references supports the use of the dyes and reagents disclosed in the references together in a single method. The three dyes and five parameters employed in Loken do not precisely discriminate erythroblasts from other blood cells. There is no indication that use of the nucleotide fluorescent dye of Kim to stain erythroblasts is at all compatible with the dyes and parameters disclosed in Loken or that any interchangeability between these may occur to one skilled in the art without further investigation or experimentation. Similarly, the dyes and reagents disclosed in Inami, along with the plotting parameters, likewise exhibit no consonance with the methods disclosed in either Loken or Kim. In sum, the incentives and motivations to combine recited by the examiner in the Office Action are based on speculation and are unsupported by fact. Therefore, the invention, as claimed, is not prima facie obvious.

Where Applicants claim 1, its sole independent claim, is clearly patentable, claims 2-13 which ultimately depend from claim 1, and thereby incorporate all limitations of the claim, are also allowable. *In re Fine*, 5 USPQ2d 1596, 1600 (Fed. Cir. 1988).

In view of the foregoing, favorable action on the merits, including entry and approval of all amendment and allowance of all claims is respectfully solicited.

Respectfully submitted,

By: _____

Leo G. Lenna
Reg. No. 42,796
BRYAN CAVE LLP
245 Park Avenue
New York, New York 10167-0034
(212) 692-1898

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